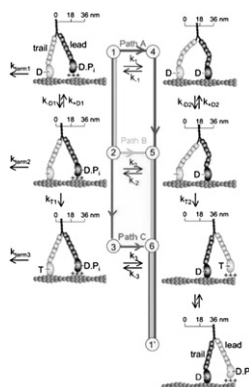


ATPase cycle (see Figure). The reduced run lengths with increasing ATP, ADP, and Pi suggest that runs terminate from two distinct states; one with both heads weakly-bound (state 3) another with ADP in the trailing head while the leading head has yet to undergo its powerstroke (state 1). In addition, to strain dependent accelerated ADP-release from the trailing head (State 4), the model also predicts that strain accelerates ATP binding (state 5) two-fold. These data and model analysis suggest that myosin Va processivity involves a complex branched kinetic pathway, providing the motor versatility when meeting the physical challenges presented by the intracellular environment.



2808-Plat

Bidirectional Cooperative Motion Of Myosin-II Motors On Actin Tracks With Randomly Alternating Polarities

Anne Bernheim, Barak Gilboa, David Gillo, Oded Farago.

Ben-Gurion University, Beer-Sheva, Israel.

The cooperative action of many molecular motors is essential for dynamic processes such as cell motility and mitosis. This action can be studied by using motility assays in which the motion of cytoskeletal filaments over a surface coated with motor proteins is tracked. In previous studies of actin-myosin II systems, fast directional motion was observed, reflecting the tendency of myosin II motors to propagate unidirectionally along actin filaments. Here, we present a motility assay with actin bundles consisting of short filamentous segments with randomly alternating polarities. These actin tracks exhibit bidirectional motion with macroscopically large time intervals (of the order of several seconds) between direction reversals. Analysis of this bidirectional motion reveals that the characteristic reversal time, τ_{rev} , does not depend on the size of the moving bundle or on the number of motors, N . This observation contradicts previous theoretical calculations based on a two-state ratchet model (Badoual *et al.* 2002. *Proc. Natl. Acad. Sci.* 99:6696-6701), predicting an exponential increase of τ_{rev} with N . We present a modified version of this model that takes into account the elastic energy due to the stretching of the actin track by the myosin II motors. The new model yields a very good quantitative agreement with the experimental results.

Platform AU: Protein-Ligand Interactions

2809-Plat

The Lysine At Position 13 Of Pten'S N-terminus Is Necessary For Its Preferred Interaction With PI(4,5)P₂

Roberta E. Redfern¹, Alexandra D. Hill¹, Alonzo Ross², Arne Gericke¹.

¹Kent State University, Kent, OH, USA, ²University of Massachusetts Medical School, Worcester, MA, USA.

Phosphatase and tensin homologue deleted on chromosome 10, also known as PTEN, has been identified as the most important regulator of the PI3K pathway, mutation or deletion of one copy of this protein results in a tumorigenic state. PTEN has also been identified as the second most important and mutated tumor suppressor, rivaled only by p53. PTEN contains within its N-terminus a PI(4,5)P₂ binding domain which has been shown to bind preferentially to PI(4,5)P₂, and whose presence within the protein is necessary for binding and activity of the enzyme. Within the PI(4,5)P₂ binding domain resides a lysine which is frequently mutated in many types of cancer, one of the most important mutations being PTEN_{K13E}. Because this mutation results in a change in the overall charge of the PI(4,5)P₂ binding domain, we have studied the effects of not only this mutation on the interaction of PTEN and its N-terminally derived peptide with PI(4,5)P₂, but have also mutated this lysine to arginine to maintain the overall charge of the binding domain, as well as moving only the position of this lysine within the N-terminus. We have found that mutation of this lysine, even those that maintain charge and overall identity of the residues within the PI(4,5)P₂ binding domain result in decreased ability to bind to PI(4,5)P₂ containing membranes. Interestingly, the proteins which have mutations at this lysine also do not undergo any conformational changes upon interaction with membranes containing PI(4,5)P₂, in contrast what was observed for the wild type protein. The lack of binding of these mutated proteins and subsequent conformational changes give insight into the mechanism of these mutations in the development of a tumorigenic state.

2810-Plat

Structural and Biophysical Characterization of the GAF Domains from Phosphodiesterases 5 and 6

Clemens C. Heikaus, Sergio E. Martinez, Gabriele Varani, Joseph A. Beavo, Rachel E. Klevit.

University of Washington, Seattle, WA, USA.

Phosphodiesterases 5 and 6 control the intracellular levels of cGMP through hydrolysis. The catalytic domains of both proteins are regulated by allosteric binding of cGMP to the N-terminal GAF domain (GAF A) of a tandem pair. We present the atomically detailed structures of both cGMP-bound GAF A domains as determined by NMR (PDE5A) and x-ray crystallography (PDE6C). Each domain adopts a conserved overall fold with well-defined cGMP binding pockets. However, the nucleotide coordination is distinct with a series of altered binding contacts. Nucleotide binding specificity is provided in each by the orientation of an Asp/Asn residue that is within hydrogen bond distance of the guanine ring. In PDE5A, a D196A mutation disrupts cGMP binding and increases cAMP affinity causing an altered cAMP-bound structural conformation in constructs containing only GAF A. NMR studies reveal that both GAF domains undergo significant cGMP-dependent conformational changes. In PDE5A, GAF B stabilizes the highly dynamic multi-state apo GAF A domain, presumably via direct interaction. In contrast, cGMP-free GAF A from PDE6C is more defined and in a single "open" state with flexible elements. Biophysical characterization of the GAF domains by Circular Dichroism and Analytical Ultracentrifugation further underlines the difference between the two PDEs. The structural features of the GAF domains from PDE5 and PDE6 revealed here provide a basis for future investigations of the regulatory mechanism of both PDEs and the design of GAF-specific small molecule inhibitors of PDE function.

2811-Plat

Mechanism Of Interaction Between The Volatile Anesthetic Halothane And A Model Ion Channel Protein: Fluorescence And Infrared Spectroscopy Employing A Cyano-phenylalanine Probe And Molecular Dynamics Simulation

Jing Liu, Hongling Zou, Joseph Strzalka, Andrey Tronin, Jonas S. Johansson, J. Kent Blasie.

University of Pennsylvania, Philadelphia, PA, USA.

We demonstrate that cyano-phenylalanine (Phe_{CN}) can be utilized to probe the binding of the inhalational anesthetic halothane to a model ion channel protein hbAP-Phe_{CN} possessing a designed binding cavity. The Trp to Phe_{CN} mutation adjacent the cavity alters neither the α -helical conformation nor the 4-helix bundle structure. The halothane binding properties of hbAP-Phe_{CN}, based on fluorescence quenching, are consistent with those of the Trp-prototype, hbAP1. The dependence of fluorescence lifetime on halothane concentration implies a one-dimensional diffusion of halothane along the nonpolar core of the protein bundle. Consequently, the fluorescence quenching is dynamic at lower halothane concentrations, becoming static at higher concentrations. The 4-helix bundle structure present in aqueous detergent solution and at the air-water interface, is preserved in multilayer films of hbAP-Phe_{CN}, enabling vibrational spectroscopy of both the protein backbone and its nitrile label (-CN). The -CN stretching vibration exhibits a largely reversible blue-shift upon halothane binding.

The complexity of this 4-helix bundle protein, where four Phe_{CN} probes are present adjacent to the designed binding site within each bundle, all contributing to the infrared absorption, requires molecular dynamics simulation to interpret the infrared results. Decomposition of the forces acting on the nitrile probes indicates that -CN's blue shift arises from the halothane induced changes in the probes' electrostatic protein environment averaged over the four probe oscillators. Although halothane remains localized within the binding cavity, it undergoes significant translational and rotational motion, modulated by the interaction of halothane's -CF₃ group with backbone hydrogen atoms of residues forming the cavity. This halothane-backbone interaction strongly outweighs the halothane-probe interaction, making -CN a good "spectator" probe of the halothane-protein interaction.

2812-Plat

Binding Kinetics of Two Hyperactive Antifreeze Proteins are Revealed by Using Novel Microfluidic Devices

Ido Braslavsky¹, Yeliz Celik¹, Peter L. Davies².

¹Ohio University, ATHENS, OH, USA, ²Queen's University, Kingston, ON, Canada.

Antifreeze proteins (AFPs) are produced by some cold-adapted organisms and function against freezing by arresting the ice crystal growth and preventing ice recrystallization. The questions regarding the binding kinetics of antifreeze proteins to ice surfaces are still a matter of debate and experimental data evaluating